

CLAIMS

We claim:

1. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising:
  - a) providing a hybridization complex comprising said target sequence and a capture probe covalently attached to a microsphere on a surface of a substrate; and
  - b) determining the nucleotide at said detection position.
- 10 2. A method according to claim 1 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.
- 15 3. A method according to claim 1 wherein said substrate is a fiber optic bundle.
4. A method according to claim 1 wherein said determining comprises:
  - a) contacting said microsphere with a plurality of detection probes each comprising:
    - i) a unique nucleotide at the readout position; and
    - ii) a unique detectable label; and
  - b) detecting a signal from at least one of said detectable labels to identify the nucleotide at the detection position.
- 20 5. A method according to claim 4 wherein said detectable labels are fluorophores.
6. A method according to claim 1 wherein said target sequence comprises a first target domain directly 5' adjacent to said detection position, wherein said hybridization complex comprises said target sequence, said capture probe and an extension primer hybridized to said first target domain of said target sequence, and said determining comprises:
  - a) contacting said microsphere with:
    - i) a polymerase enzyme;
    - ii) a plurality of NTPs each comprising a covalently attached detectable label; under conditions whereby if one of said NTPs basepairs with the base at said detection position, said extension primer is extended by said enzyme to incorporate said label; and
  - c) identifying the base at said detection position.
- 30 35 7. A method according to claim 6 wherein said label is a fluorophore.
8. A method according to claim 7 wherein each NTP comprises a unique fluorophore.

9. A method according to claim 6 wherein said label comprises biotin.

10. A method according to claim 9 wherein said label comprises imine-biotin.

5 11. A method according to claim 6 wherein said label comprises a functional group for addition of a fluorophore.

10 12. A method according to claim 1 wherein said target sequence comprises a first target domain directly 5' adjacent to said detection position, wherein said capture probe serves an extension primer and is hybridized to said first target domain of said target sequence, and said determining comprises:

a) contacting said microsphere with:

i) a polymerase enzyme;

15 ii) a plurality of NTPs each comprising a covalently attached detectable label; under conditions whereby if one of said NTPs basepairs with the base at said detection position, said extension primer is extended by said enzyme to incorporate said label; and

c) identifying the base at said detection position.

13. A method for according to claim 1 wherein said target sequence comprises 5' to 3':

20 a) a first target domain comprising an overlap domain comprising at least a nucleotide in the detection position; and

b) a second target domain contiguous with said detection position;

wherein said hybridization complex comprises:

a) a first probe hybridized to said first target domain; and

25 b) a second probe hybridized to said second target domain, wherein said second probe comprises:

i) a detection sequence that does not hybridize with said target sequence; and

ii) a detectable label;

wherein if said second probe comprises a base that is perfectly complementary to said detection position a cleavage structure is formed;

30 said method further comprising:

a) contacting said hybridization complex with a cleavage enzyme that will cleave said detection sequence;

d) forming an assay complex with said detection sequence, a capture probe covalently attached to a microsphere on a surface of a substrate, and at least one label;

35 e) detecting the presence or absence of said label as an indication of the formation of said cleavage structure; and

f) identifying the base at said detection position.

14. A method according to claim 13 wherein said label comprises a fluorophore.

15. A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, said method comprising:

- a) hybridizing a first ligation probe to said first target domain;
- b) hybridizing a second ligation probe to said second target domain, wherein if said second ligation probe comprises a base that is perfectly complementary to said detection position a ligation structure is formed;
- c) providing a ligation enzyme that will ligate said first and said second ligation probes to form a ligated probe;
- d) forming an assay complex with said ligated probe, a capture probe covalently attached to a microsphere on a surface of a substrate, and at least one label;
- e) detecting the presence or absence of said label as an indication of the formation of said ligation structure; and
- f) identifying the base at said detection position.

16. A method according to claim 15 wherein said label is a fluorophore.

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